

- d) projecting a three-dimensional coordinate of said light scatter signals from each cell onto a precalibrated three dimensional surface containing grid lines of V and HC;
- e) determining the values of V and HC by the location of each projected intercept onto said three dimensional grid surface.

12. A three-dimensional optical method for determining the volume, V, and hemoglobin content, HC, of individual red blood cells, said method comprising the steps of;

- a) treating an anti-coagulated whole blood sample with a reagent solution, said solution comprising a sphering agent and a neutrally buffered isotonic saline solution,;
- b) passing red blood cells of said sample in single file through a light a light beam directed along an optical path at a selected wavelength;
- c) measuring the resultant magnitude of one forward angle light scatter signal, one light loss signal, and a third side-angle light scatter signal from each cell;
- d) projecting a three-dimensional coordinate of said light scatter signals from each cell onto a pre-calibrated three dimensional surface containing grid lines of V and HC;
- e) determining the values of V and HC by the location of each projected intercept onto said three dimensional grid surface.